Effects of Cell Surface Charge and Hydrophobicity on Attachment of 16 *Salmonella* Serovars to Cantaloupe Rind and Decontamination with Sanitizers†

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ABSTRACT

Adherence of bacteria to cantaloupe rind is favored by surface irregularities such as roughness, crevices, and pits, thus reducing the ability of washing or sanitizer treatments to remove or inactivate attached cells. In this study, we compared the surface charge and hydrophobicity of two cantaloupe-related outbreak strains of *Salmonella* Poona (RM2350 and G-91-1595) to those of 14 additional *Salmonella* strains using electrostatic and hydrophobic interaction chromatography. The relative abilities of the 16 strains to attach to cantaloupe surfaces and resist removal by washing with water, chlorine (200 ppm), or hydrogen peroxide (2.5%) for 5 min after a storage period of up to 7 days at 5 to 20°C also were determined. Whole cantaloupes were inoculated with each pathogen at 8.36 log CFU/ml, dried for 1 h inside a biosafety cabinet, stored, and then subjected to the washing treatments. Only the positive surface charge of the two cantaloupe-related strains of *Salmonella* Poona was significantly higher ($P < 0.05$) than that of the other strains. Initial bacterial attachment to cantaloupe surfaces ranged from 3.68 to 4.56 log CFU/cm^2 (highest values for *Salmonella* Michigan, Newport, Oranienburg, and Mbandaka). The average percentage of the total bacterial population strongly attached to the cantaloupe surface for the *Salmonella* serovars studied ranged from 0.893 to 0.946 at 5°C and from 0.987 to 0.999 at 25°C. Washing inoculated melons with water did not produce a significant reduction in the concentration of the pathogens ($P > 0.05$). Chlorine and hydrogen peroxide treatments caused an average 3-log reduction when applied 20 to 40 min postinoculation. However, sanitizer treatments applied 60 min or more postinoculation were less effective (approximately 2.5-log reduction). No significant differences were noted in sanitizer efficacy against the individual strains ($P > 0.05$). The two cantaloupe-related outbreak *Salmonella* Poona strains did not significantly differ from the other *Salmonella* strains tested in negative cell surface charge or hydrophobicity, were not more effective in attaching to whole melon surfaces, and were not more resistant to the various washing treatments when present on rinds.

For most consumers, fresh-cut cantaloupe melon is a refreshing and healthy dessert or snack. However, outbreaks of salmonellosis associated with consumption of contaminated melons may lead to reductions in sales and consumer confidence in the safety of the product. Seven melon-related salmonellosis outbreaks involving hundreds of cases have been reported since 1990, and pathogen-contaminated domestic and imported melons have been detected *(6, 25–28)*. Although the largest melon-related outbreaks have been attributed to various *Salmonella* serotypes (including *Salmonella* Poona), other human pathogens such as *Escherichia coli* O157:H7, *Campylobacter jejuni*, and Norwalk-like virus also have been implicated *(9)*. Salmonellosis outbreaks in 2000 through 2002 were traced by the U.S. Food and Drug Administration (FDA) to melons imported from Mexico. From on-farm investigations conducted in Mexico, the FDA concluded that "measures were not in place to minimize microbial contamination in growing, harvesting, packaging, and cooling of cantaloupe" *(28)*. There is an urgent need for improved control strategies for eliminating or reducing populations of human pathogens on the surfaces of minimally processed melons *(7)*.

Several chemical and physical methods have been reported for decontamination of melon before fresh-cut processing, but none of these methods eliminates human pathogens attached on the melon surface *(2, 20, 22–24)*. The raised net tissue of whole cantaloupes gives the surface an inherent roughness that favors microbial attachment and complicates detachment. Information concerning the ecology of human pathogens on melons is lacking. It is not known whether human pathogens adhere to melon surfaces via bacterial fimbriae or other bacterial surface components such as exopolysaccharides nor how quickly the attachment process occurs. The state of human pathogens on the surfaces of fruits and vegetables may have profound implications for their susceptibility to antimicrobial treatments. Ukuku and Fett *(21)* reported that both charge and hydrophobicity influence attachment of bacterial pathogen cells to cantaloupe rind. In that study, the strength of attachment to the rind was only determined at postinoculation day 0 (at 6 h) and after 3 to 7 days of storage at 4°C. Bacterial cell surface hydrophobicity was identified as an important factor in cell attachment and immobilization in the intercellular spaces of soybean leaves *(12)*. The aims of this

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TABLE 1. Salmonella *strains used in this study*[a]

Serovar	Strain	Origin	Source[b]
Anatum	F4317	Sprout-related outbreak	A
Gaminara	02-615	Cantaloupe	B
Hildalgo	02-517-2	Cantaloupe	B
Infantis	F4319	Sprout-related outbreak	A
Mbandaka	00-916-1	Cantaloupe	B
Michigan		Cantaloupe-related outbreak	C
Newport	H1275	Sprout-related outbreak	A
Oranienburg	389	Cantaloupe	B
Poona	RM2350 (CA DHS 00A3563)	Cantaloupe-related outbreak	D
Poona	G91-1595	Cantaloupe-related outbreak	E
Poona	953	Ovine meat and bone meal	B
Poona	348	Cantaloupe	B
Poona	418	Octopus	B
St. Paul	02-517	Cantaloupe	B
Stanley	H0558	Sprout-related outbreak	A
Typhimurium	45	Cantaloupe	B

[a] Individual mean inoculum was 8.6 ± 0.36 log CFU/ml.

[b] A, P. Griffin, Centers for Disease Control and Prevention, Atlanta, Ga.; B, D. Farmer, U.S. Food and Drug Administration, Jefferson, Ark.; C, L. R. Beuchat, Center for Food Safety, University of Georgia, Griffin; D, R. Mandrell, ARS Western Regional Research Center, Albany, N.Y.; E, F. T. Leano, Minnesota Department of Health, Minneapolis.

study were (i) to investigate how rapidly *Salmonella* cells attach to melon rind, (ii) to determine whether differences in the ability to attach to cantaloupe surface exist among *Salmonella* serovars, including cantaloupe and non–cantaloupe-related strains, and (iii) to determine the relationship between bacterial cell surfaces and hydrophobicity and bacterial cell attachment or resistance to washing and sanitizer treatments.

MATERIALS AND METHODS

Sixteen *Salmonella* strains representing 12 serovars of various origins were used in this study (Table 1). Bacteria were maintained on brain heart infusion agar (BBL/Difco, Becton Dickinson, Sparks, Md.) slants held at 4°C. Before use, each culture was subjected to two successive transfers by loop inoculation to 5 ml of brain heart infusion broth (BBL/Difco, Becton Dickinson). A final transfer of 0.2 ml was made into 20 ml of brain heart infusion broth, and the culture was incubated at 36°C for 18 h under static conditions. Bacterial cells were harvested by centrifugation (10,000 × *g* for 10 min) at 4°C, and the cell pellets were washed in salt-peptone (0.85% NaCl and 0.05% Bacto Peptone; BBL/Difco, Becton Dickinson). Cell pellets were used to prepare two different types of inoculum. The first inoculum type consisted of individual bacterial strains at 1.9×10^8 CFU/ml. The second inoculum type consisted of a mixture containing five strains of *Salmonella* Poona at approximately 1.4×10^8 CFU/ml per strain. All inocula were prepared in 3 liters of 0.1% (wt/vol) peptone water.

Chromatography. Hydrophobic interaction chromatography (HIC) and electrostatic interaction chromatography (ESIC) columns were prepared according the procedure described by Dahlback et al. *(8)* and Pedersen *(16)* with slight modification. The ESIC columns used were Poly-Prep prefilled chromatography columns chloride form (anionic, AG 1-X 8 Resin, 100 to 200 mess, 0.8 by 4 cm) and hydrogen form (cationic, AG 50-X 8 Resin, 100 to 200 mess, 0.8 by 4 cm; Bio-Rad Laboratories, Hercules, Calif.). For the HIC, columns were packed with 12 ml of octyl-Sepharose CL-4B gel (Sigma, St. Louis, Mo.) and equilibrated overnight at 4°C in 18 ml of 0.02 M $NaPO_4$ buffer, pH 6.8 (bed volume of 0.8 ml). Chromatography was conducted according to Dickson and Koohmaraie *(11)*. A sample (0.1 ml) of washed bacterial cell suspension (1.9×10^8 CFU/ml) for each strain was loaded onto the surface of the column followed by 0.2 ml of $NaPO_4$ buffer. The bacterial cell populations in the suspensions added to the column were determined by standard dilution plating techniques. Elution of all columns was performed with 12 ml of 0.02 M $NaPO_4$ buffer, pH 6.8, and the eluate was collected. Bacterial populations in each eluate sample and in the original suspensions were determined using xylose lysine tergitol 4 (XLT4) agar (Difco/BBL, Becton Dickinson) and a pour-plate technique. The number of bacteria bound to the columns was calculated as the difference between the total cell population in the initial suspension and in the eluted samples. The relative hydrophobicity was expressed as *g/e* and the relative ion values were expressed as *r/e*, where *g* and *r* represent the number of bacteria retained by the columns and *e* represents the numbers eluted. Each strain was tested three times.

Inoculation of cantaloupe. Unwaxed whole cantaloupes (Western shippers) purchased from a local produce distributor were left at room temperature (~20°C) overnight before being inoculated. Individual cantaloupes were submerged in 3 liters of each inoculum at ~18°C (a total of two cantaloupes per 3 liters) and agitated by stirring with a gloved hand for 10 min before storage (0 to 168 h) at 5 or 25°C for further treatment and microbial studies.

Washing treatments. A commercial bleach solution of 5.25% sodium hypochlorite (NaOCl; Clorox, Clorox Company, Oakland, Calif.) was diluted in sterile water to obtain a wash solution containing 200 ppm of free chlorine. The pH was adjusted to 6.4 ± 0.1 by adding citric acid (Sigma). Free chlorine in the solution was determined with a chlorine test kit (Hach Co., Ames, Iowa). A second wash solution containing 2.5% hydrogen peroxide was prepared from a 30% stock solution of hydrogen peroxide (reagent grade, Fisher Scientific, Suwanee, Ga.) by dilution

with sterilized tap water. Washing treatments were performed by totally submerging the melons in 3 liters of sterile tap water, 200 ppm chlorine, or 2.5% hydrogen peroxide. Melons were manually rotated for 5 min to assure complete contact of surfaces with the wash solution. Washed melons were placed on crystallizing dishes inside a biosafety cabinet to dry for 1 h.

Attachment study. For the attachment study, inoculated melons stored for 20 min, 30 min, 1 h, 2 h, 6 h, 9 h, 1 day, 3 days, and 7 days were washed with water. The numbers of bacterial cells in the wash water and remaining on the melon surfaces were then determined. The population remaining on the melon surface after the washing treatment was expressed in terms of the S_R value, which represents the percentage of the total bacterial population strongly attached to the cantaloupe surface (remaining on the surface after washing with water). S_R values were calculated as the number of strongly attached bacteria divided by the number of loosely plus strongly attached bacteria, as reported by Dickson and Koohmaraie *(11)*.

Sample preparation and enumeration of *Salmonella*. A sterilized stainless steel cork borer was used to cut rind plugs at random locations on the cantaloupe surface to produce 152 plugs per cantaloupe. Each plug was 22 mm in diameter with a rind surface area (πr^2) of 3.80 cm^2; 72 plugs were randomly selected and further processed. The flesh adhering to the rind plugs was trimmed off with a sterilized stainless steel knife. The plugs were blended at speed five for 1 min in a Waring Blendor with 75 ml of 0.1% peptone water. Decimal dilutions of the samples were made with 0.1% peptone water, and 0.1 ml was plated in duplicate on XLT4 agar and incubated at 35°C for 48 h. For the wash water, estimates of *Salmonella* populations in 1-ml samples of wash water were obtained using the pour-plate method with XLT4 agar incubated at 35°C for 48 h.

Preparation of fresh-cut melon pieces. All utensils and equipment used for preparing fresh-cut melon pieces were sanitized with 200 ppm chlorinated water (prepared from sodium hypochlorite with pH adjusted to 6.4 with citric acid). Treated and untreated whole melons were cut into four sections with a sterile knife, and the rinds were carefully removed. Fresh-cut pieces (~3-cm cubes) from multiple treated melons were pooled, placed inside three-pocket Tub Tall plastic bowls (9.75 in. [24.75 cm] in diameter; Mach 2, Rock-Tenn Company, Franklin Park, Ill.), and stored at 5, 10, 15, and 20°C. For microbiological analyses, 100 g of fresh-cut cubes were homogenized in 200 ml of 0.1% peptone water by pummeling in a Stomacher 400 (Dynatech Laboratories, Alexandria, Va.) for 30 s at medium speed. Samples were serially diluted and plated as stated above to determine populations of *Salmonella* every 2 days for up to 10 days at each storage temperature. For comparison, a pure culture of *Salmonella* Poona RM 2350 was plated on XLT4 agar and evaluated in parallel with the samples. Selected black or black-centered colonies from the agar plates were confirmed to be *Salmonella* according to the FDA *Bacteriological Analytical Manual* following conventional biochemical methods *(1)* and latex agglutination serological assays (Oxoid, Ogdensburg, N.Y.).

Data analysis. All experiments were done in triplicate, and duplicate samples were analyzed at each sampling time. Values are reported as the mean and standard deviation (SD). Data were subjected to analysis of variance using the Statistical Analysis System Program (SAS Institute, Cary, N.C.). Differences between mean values were evaluated for significance ($P < 0.05$) with the Bonferroni least significant difference method *(15)*.

TABLE 2. *Cell surface charge and hydrophobicity of 16* Salmonella *strains*[a]

Salmonella strain	Hydrophobicity	ESIC charge	
		$-r/e$	$+r/e$
Anatum	0.421 ± 0.110	18.98 ± 0.44	ND
Gaminara	0.419 ± 0.103	19.42 ± 0.53	ND
Hildalgo	0.428 ± 0.121	17.95 ± 0.35	ND
Infantis	0.399 ± 0.114	18.98 ± 0.23	ND
Mbandaka	0.461 ± 0.121	47.38 ± 0.62	ND
Michigan	0.651 ± 0.113	68.45 ± 1.27	ND
Newport	0.648 ± 0.157	63.28 ± 1.46	ND
Oranienburg	0.448 ± 0.109	57.72 ± 1.25	ND
Poona G-91-1595	0.446 ± 0.105	35.16 ± 0.36	4.31 ± 0.35
Poona RM2350	0.633 ± 0.163	30.18 ± 0.43	6.29 ± 0.49
Poona 953	0.398 ± 0.074	27.89 ± 0.22	ND
Poona 348	0.456 ± 0.115	38.88 ± 0.46	ND
Poona 418	0.378 ± 0.083	34.19 ± 0.49	ND
St. Paul 02-517	0.356 ± 0.086	21.86 ± 0.52	ND
Stanley H0558	0.495 ± 0.155	18.98 ± 1.13	2.19 ± 0.18
Typhimurium 045	0.441 ± 0.133	22.72 ± 0.72	ND

[a] Values are means ± SD of three experiments, with duplicate determinations per experiment. ND, not determined in the eluate.

RESULTS

Relative bacterial cell surface hydrophobicity and charge. Surface hydrophobicity was variable among the 16 *Salmonella* strains (Table 2). *Salmonella* Michigan, *Salmonella* Poona RM2350, and *Salmonella* Newport strains had the highest cell surface hydrophobicity (g/e = 0.651, 0.633, and 0.648, respectively) followed by the rest with g/e values averaging 0.42. All of the bacteria tested had higher negative surface charges than positive surface charges (Table 2). *Salmonella* Michigan, *Salmonella* Newport, and *Salmonella* Oranienburg had the highest negative surface charges, with r/e values of 68.45, 63.28, and 57.72, respectively. Only the positive surface charge of the two cantaloupe-related outbreak strains of *Salmonella* Poona were significantly higher ($P < 0.05$) than those of the other strains.

Attachment of individual *Salmonella* strains to cantaloupe surfaces. The initial populations of individual *Salmonella* strains inoculated onto cantaloupe surfaces ranged from 3.97 to 4.98 log CFU/cm^2. *Salmonella* Michigan had the highest initial number of attached bacteria on the melon surface (4.98 log CFU/cm^2), followed by *Salmonella* St. Paul (4.67 log CFU/cm^2) and *Salmonella* Newport (4.55 log CFU/cm^2). Populations for the rest of the strains ranged from 3.97 to 4.50 log CFU/cm^2.

The S_R values for individual *Salmonella* strains attached on cantaloupe surfaces determined at 30 min to 2 h after inoculation and after washing with water were not significantly different (data not shown). The S_R values for individual *Salmonella* strains attached on cantaloupe surfaces determined at 24 h to 7 days after inoculation, storage

TABLE 3. *Effect of chlorine and hydrogen peroxide on log reduction of* Salmonella *strains on whole cantaloupe surfaces after storage of melons at 5°C for 30 min*

Salmonella strain	*Salmonella* concn (log CFU/cm^2)[a]			
	Control	Water	200 ppm chlorine	2.5% H_2O_2
Anatum	4.14 ± 0.21 A	2.16 ± 0.12 C	3.00 ± 0.19 B	3.02 ± 0.14 B
Gaminara	4.79 ± 0.20 A	2.01 ± 0.18 C	2.67 ± 0.16 B	2.55 ± 0.16 B
Hildalgo	3.79 ± 0.19 A	2.04 ± 0.13 C	3.00 ± 0.15 B	3.13 ± 0.15 B
Infantis	4.18 ± 0.24 A	2.16 ± 0.15 C	3.00 ± 0.14 B	3.21 ± 0.13 B
Mbandaka	4.00 ± 0.22 A	2.00 ± 0.15 C	2.58 ± 0.18 B	2.70 ± 0.15 B
Michigan	4.85 ± 0.19 A	1.62 ± 0.15 C	2.04 ± 0.19 B	2.18 ± 0.17 B
Newport	4.48 ± 0.15 A	1.74 ± 0.16 C	2.62 ± 0.15 B	2.64 ± 0.16 B
Oranienburg	4.05 ± 0.16 A	1.95 ± 0.17 C	2.48 ± 0.14 B	2.57 ± 0.16 B
Poona G-91-1595	4.00 ± 0.16 A	2.14 ± 0.14 C	3.00 ± 0.12 B	3.00 ± 0.17 B
Poona RM2350	4.26 ± 0.16 A	2.00 ± 0.18 C	2.39 ± 0.18 B	2.56 ± 0.13 B
Poona 953	4.00 ± 0.20 A	2.04 ± 0.15 C	3.00 ± 0.15 B	3.00 ± 0.19 B
Poona 348	4.00 ± 0.21 A	2.04 ± 0.16 C	3.03 ± 0.14 B	3.07 ± 0.14 B
Poona 418	4.00 ± 0.17 A	2.01 ± 0.16 C	2.94 ± 0.16 B	3.02 ± 0.15 B
St. Paul 02-517	4.52 ± 0.17 A	2.01 ± 0.17 C	2.74 ± 0.13 B	2.82 ± 0.20 B
Stanley H0558	4.16 ± 0.21 A	2.00 ± 0.16 C	2.88 ± 0.14 B	2.86 ± 0.17 B
Typhimurium 045	4.38 ± 0.16 A	2.00 ± 0.15 C	2.84 ± 0.17 B	2.92 ± 0.13 B

[a] Values are means ± SD of three experiments, with duplicate determinations per experiment. Within each column and each row, means not followed by the same letter are significantly different ($P < 0.05$).

at 5 and 25°C, and washing with water also were not significantly different (data not shown). Attachment strength for all cantaloupe-related strains with the exception of *Salmonella* Michigan were not significantly different ($P < 0.05$) than that for non–cantaloupe-related strains.

Attachment of *Salmonella* Poona to cantaloupe surfaces and sanitizer efficacy. The initial attachment of all 16 *Salmonella* strains to the surface of cantaloupes stored at 5°C for 30 min postinoculation is shown in Table 3. Washing with water within 30 min after inoculation led to removal of 1.8 log CFU/cm^2 of the *Salmonella* Poona cocktail from the melon surface. Sanitizer efficacy was not significantly different ($P > 0.05$) when applied at 30 min postinoculation to melons stored at 25°C (Table 4). Populations of all *Salmonella* strains on surfaces of control cantaloupes stored at 5°C were slightly lower but were not significantly different ($P > 0.05$) (Fig. 1). Regardless of storage temperature (5 or 25°C) or time of water washing treatment, the S_R values for all *Salmonella* strains were the same (0.999) when tested at 20 min or 7 days postinoculation (data not shown). The S_R values for *Salmonella* Michigan, *Salmonella* St. Paul, and *Salmonella* Newport were significantly different ($P < 0.05$) from those for all the *Salmonella* Poona strains. However, of the *Salmonella* Poona strains, strain 418 from octopus had the highest S_R values at 24 h or longer. The S_R values for all *Salmonella* strains from the sprout-related outbreak were not significantly different except that for *Salmonella* Newport. Population reduction of *Salmonella* by sanitizer treatment also was not significantly different ($P > 0.05$) between cantaloupe-related and sprout-related outbreak strains inoculated onto surfaces of melons stored at 25°C for 120 min (Fig. 2). Efficacy of sanitizer treatments with chlorine (200 ppm) or hydrogen peroxide (2.5%) at 40 min after inoculation was reduced and produced an approximately 3-log reduction in *Salmonella* concentrations on the cantaloupe surface. With longer postinoculation storage, populations gradually decreased by 2.5 log CFU/cm^2 at 24 h (Tables 5 and 6). Differences in efficacy between the two sanitizers were not significant ($P > 0.05$). Log reductions for all strains showed similar trends at 24 h or longer, and by 168 h of storage populations of *Salmonella* Michigan, *Salmonella* Typhimurium, *Salmonella* Poona, and *Salmonella* Newport were reduced by 2.3 log CFU/cm^2.

Transfer of *Salmonella* Poona to fresh-cut melon pieces. The populations of a five-strain cocktail of *Salmonella* Poona on fresh-cut melon pieces prepared from melons sanitized in 200 ppm chlorine at 2 h or 7 days postinoculation and then stored for various times and temperatures are shown in Figure 3. No *Salmonella* was recovered on fresh-cut melon pieces prepared immediately from melons sanitized at 2 h and 168 h postinoculation and during storage at 5°C for 8 and 2 days, respectively. The population of *Salmonella* recovered on fresh-cut pieces was greater when inoculated melons were stored longer than 2 h before sanitizing and processing. Growth was detected earlier on fresh-cut pieces prepared from inoculated melons held 7 days prior to sanitizing and processing compared with fresh-cut pieces prepared after 2 h of storage at 5, 10, 15, or 20°C. This finding suggests greater transfer of *Salmonella* from melon surface to fresh-cut pieces with the longer postinoculation storage time before sanitizer treatment.

DISCUSSION

The outer surface (rind) of a cantaloupe has a variety of surfaces to which a bacterial cell may bind. The epidermal cell surface is ruptured by a meshwork of raised tissue

TABLE 4. *Effect of chlorine and hydrogen peroxide on log reduction of* Salmonella *strains on whole cantaloupe surfaces after storage of melons at 25°C for 30 min*

Salmonella strain	*Salmonella* concn (log CFU/cm²)			
	Control	Water	200 ppm chlorine	2.5% H_2O_2
Anatum	4.38 ± 0.24 A	2.38 ± 0.22 C	3.19 ± 0.14 B	3.22 ± 0.16 B
Gaminara	4.98 ± 0.21 A	2.01 ± 0.21 C	2.89 ± 0.18 B	2.92 ± 0.17 B
Hildalgo	3.98 ± 0.21 A	2.24 ± 0.15 C	3.24 ± 0.14 B	3.22 ± 0.19 B
Infantis	4.25 ± 0.24 A	2.36 ± 0.17 C	3.14 ± 0.13 B	3.30 ± 0.15 B
Mbandaka	4.13 ± 0.31 A	2.00 ± 0.15 C	2.84 ± 0.14 B	2.92 ± 0.15 B
Michigan	4.98 ± 0.17 A	1.98 ± 0.19 C	2.44 ± 0.17 B	2.38 ± 0.13 B
Newport	4.55 ± 0.16 A	2.04 ± 0.13 C	2.84 ± 0.17 B	2.88 ± 0.15 B
Oranienburg	4.19 ± 0.21 A	2.01 ± 0.14 C	2.64 ± 0.16 B	2.75 ± 0.14 B
Poona G-91-1595	4.00 ± 0.23 A	2.34 ± 0.14 C	3.14 ± 0.14 B	3.12 ± 0.15 B
Poona RM2350	4.49 ± 0.19 A	2.00 ± 0.18 C	2.64 ± 0.16 B	2.72 ± 0.15 B
Poona 953	4.16 ± 0.23 A	2.24 ± 0.17 C	3.22 ± 0.13 B	3.22 ± 0.16 B
Poona 348	4.18 ± 0.22 A	2.34 ± 0.14 C	3.33 ± 0.12 B	3.27 ± 0.16 B
Poona 418	4.28 ± 0.20 A	2.24 ± 0.15 C	3.24 ± 0.14 B	3.22 ± 0.19 B
St. Paul 02-517	4.68 ± 0.14 A	2.31 ± 0.12 C	3.14 ± 0.16 B	3.12 ± 0.21 B
Stanley H0558	4.38 ± 0.24 A	2.28 ± 0.15 C	3.16 ± 0.16 B	3.19 ± 0.15 B
Typhimurium 045	4.52 ± 0.19 A	2.04 ± 0.13 C	3.04 ± 0.16 B	3.12 ± 0.16 B

[a] Values are means ± SD of three experiments, with duplicate determinations per experiment. Within each column and each row, means not followed by the same letter are significantly different ($P < 0.05$).

(the net), which consists of lenticels and phellum (cork) cells. These cells have hydrophobic suberized walls to reduce water loss and protect against pathogen ingress. The cuticle, which is composed of waxes and cutin, covers the epidermal cells and is responsible for the hydrophobic nature of the outer surface of the cantaloupe *(29)*. The mechanisms of attachment of bacterial cells to plant surfaces have been studied most extensively for plant pathogens and symbionts *(17)*. Flagella, fimbriae, outer membrane proteins, and extracellular polysaccharides have all been implicated in attachment. In contrast, there is little information available on the attachment of bacterial human pathogens to plant surfaces. In this study, the differences in log reduction of *Salmonella* on melons stored at 5 and 25°C could be attributed to the effect of low-temperature storage, which may have affected the bacterial outer membrane proteins and possibly the production of extracellular polysaccharides. *Salmonella* can produce the extracellular carbohydrate polymer cellulose, and cellulose and curli (aggregative fimbriae), as the two principle components of the extracellular matrix, are thought to be responsible for biofilm formation *(4, 30)*. Cellulose production and the presence of curli may allow for strong attachment of *Salmonella* cells to the cantaloupe rind.

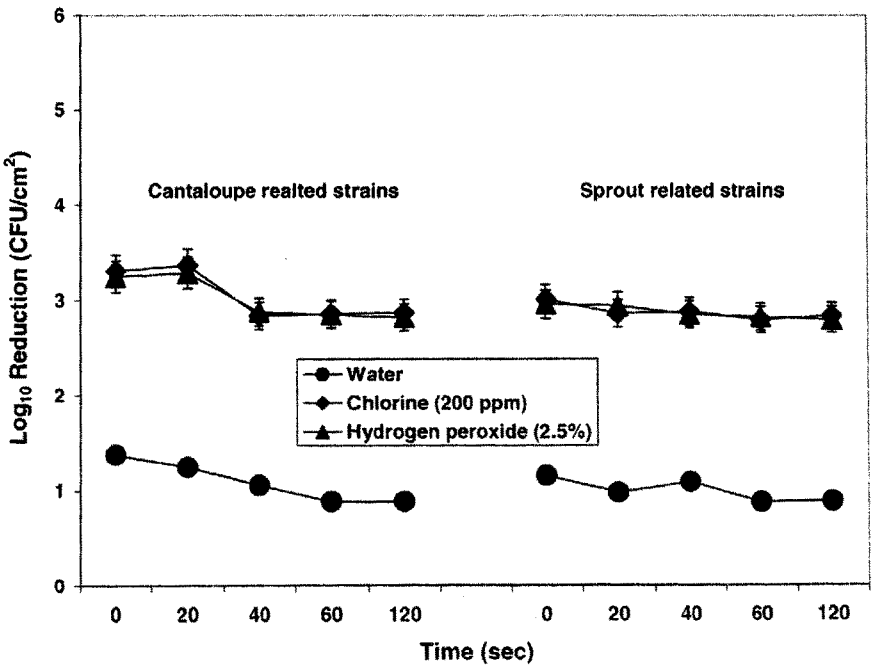

FIGURE 1. *Efficacy of sanitizing treatments on melons that had been inoculated with a cocktail of cantaloupe-related (A) and sprout-related (B)* Salmonella *strains and stored at 5°C. Values are mean ± SD of three experiments, with duplicate determinations per experiment.*

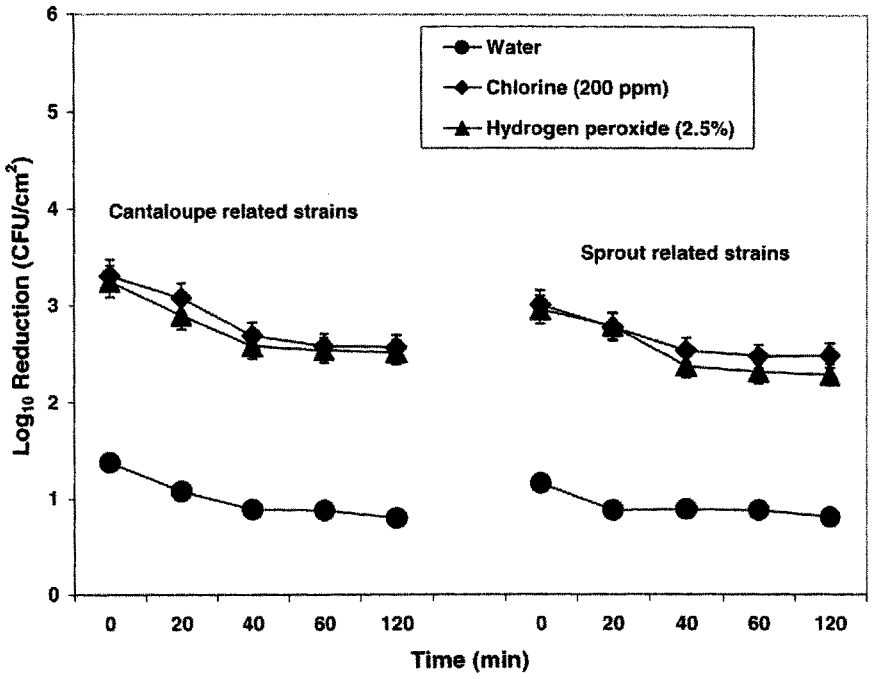

FIGURE 2. *Efficacy of sanitizing treatments on melons that had been inoculated with a cocktail of cantaloupe-related (A) and sprout-related (B)* Salmonella *strains and stored at 25°C. Values are mean ± SD of three experiments, with duplicate determinations per experiment.*

The greater transfer of bacteria from the surface to the flesh that occurred when the sanitizer treatment was applied at 7 days compared with 2 h postinoculation mostly was due to a lowered efficacy of the sanitizer treatments because of stronger attachment and possibly biofilm formation of the pathogens. Previously, we reported a relationship between cell surface charge or hydrophobicity of *Salmonella, E. coli* O157:H7, and *Listeria monocytogenes* and the strength of attachment of these pathogen cells to cantaloupe rind *(26)*. In that study, we investigated only three serovars of each pathogen and strength of attachment only on days 0 (6 h), 3, and 7. In the current study, 16 strains of *Salmonella* representing 12 serovars were investigated to determine how rapidly strength of attachment increases (minutes versus hours) and to define the relationship between strength of attachment and sanitizer efficacy. Five strains of *Salmonella* Poona (including three cantaloupe-related strains) were included because recent outbreaks of salmonellosis due to consumption of contaminated cantaloupe were associated with this serovar *(25, 26)*. It is difficult to predict the surface properties of bacterial human pathogens when the pathogens are first exposed to a plant surface

TABLE 5. *Reduction of* Salmonella *on whole cantaloupe surfaces after storage of melons at 5°C for up to 168 h after treatment with chlorine (200 ppm, 2 min)*

	Salmonella concn (log CFU/cm^2)[a]		
Salmonella strain	24 h	72 h	168 h
Anatum	2.34 ± 0.18 AX	2.38 ± 0.14 AX	2.32 ± 0.15 AX
Gaminara	2.44 ± 0.14 AX	2.46 ± 0.16 AX	2.42 ± 0.16 AY
Hildalgo	2.86 ± 0.15 AX	2.79 ± 0.14 AX	2.88 ± 0.22 AY
Infantis	2.54 ± 0.20 AX	2.56 ± 0.16 AY	2.52 ± 0.15 AX
Mbandaka	2.50 ± 0.14 AX	2.53 ± 0.13 AX	2.48 ± 0.16 AY
Michigan	2.16 ± 0.16 BX	2.12 ± 0.15 BY	2.08 ± 0.16 BY
Newport	2.43 ± 0.12 BX	2.46 ± 0.20 AX	2.39 ± 0.20 BY
Oranienburg	2.50 ± 0.13 AX	2.49 ± 0.13 AY	2.53 ± 0.15 AY
Poona G-91-1595	2.63 ± 0.15 AX	2.56 ± 0.16 AX	2.38 ± 0.20 AY
Poona RM2350	2.57 ± 0.14 AX	2.42 ± 0.17 AX	2.35 ± 0.16 AY
Poona 953	2.65 ± 0.16 AX	2.62 ± 0.14 AY	2.68 ± 0.22 AX
Poona 348	2.55 ± 0.14 AX	2.53 ± 0.15 AX	2.50 ± 0.18 AY
Poona 418	2.53 ± 0.15 AX	2.52 ± 0.16 AX	2.38 ± 0.14 BY
St. Paul 02-517	2.45 ± 0.20 AX	2.40 ± 0.20 AY	2.38 ± 0.15 BY
Stanley H0558	2.35 ± 0.14 AX	2.38 ± 0.21 BY	2.32 ± 0.18 BY
Typhimurium 045	2.29 ± 0.12 BX	2.30 ± 0.14 BX	2.19 ± 0.15 BY

[a] Values are means ± SD of three experiments, with duplicate determinations per experiment. Control for each strain averaged 4.20 log CFU/cm^2. In columns (A or B) and rows (X or Y), means not followed by the same letter are significantly different ($P < 0.05$).

TABLE 6. *Reduction of* Salmonella *on whole cantaloupe surfaces after storage of melons at 25°C for up to 168 h after treatment with chlorine (200 ppm, 2 min)*

Salmonella strain	*Salmonella* concn (log CFU/cm²)[a]		
	24 h	72 h	168 h
Anatum	2.84 ± 0.18 AX	2.66 ± 0.18 AX	2.74 ± 0.18 AX
Gaminara	2.76 ± 0.24 AX	2.58 ± 0.17 AX	2.48 ± 0.14 AY
Hildalgo	3.16 ± 0.18 AX	2.76 ± 0.16 AX	2.68 ± 0.25 AY
Infantis	2.84 ± 0.21 AX	2.54 ± 0.16 AY	2.72 ± 0.12 AX
Mbandaka	2.70 ± 0.16 AX	2.55 ± 0.15 AX	2.40 ± 0.19 AY
Michigan	2.59 ± 0.20 BX	2.22 ± 0.13 BY	2.28 ± 0.18 BY
Newport	2.60 ± 0.12 BX	2.51 ± 0.22 AX	2.29 ± 0.22 BY
Oranienburg	2.83 ± 0.18 AX	2.65 ± 0.13 AY	2.68 ± 0.14 AY
Poona G-91-1595	2.88 ± 0.12 AX	2.46 ± 0.12 AX	2.41 ± 0.22 AY
Poona RM2350	3.03 ± 0.12 AX	2.72 ± 0.21 AX	2.54 ± 0.20 AY
Poona 953	2.87 ± 0.17 AX	2.57 ± 0.16 AY	2.62 ± 0.20 AX
Poona 348	2.85 ± 0.15 AX	2.69 ± 0.12 AX	2.49 ± 0.15 AY
Poona 418	2.83 ± 0.12 AX	2.62 ± 0.21 AX	2.28 ± 0.18 BY
St. Paul 02-517	2.75 ± 0.26 AX	2.48 ± 0.23 AY	2.35 ± 0.12 BY
Stanley H0558	2.85 ± 0.18 AX	2.31 ± 0.23 BY	2.35 ± 0.18 BY
Typhimurium 045	2.59 ± 0.22 BX	2.34 ± 0.12 BX	2.29 ± 0.16 BY

[a] Values are means ± SD of three experiments, with duplicate determinations per experiment. Control for each strain averaged 4.70 log CFU/cm². In columns (A or B) and rows (X or Y), means not followed by the same letter are significantly different ($P < 0.05$).

because environmental conditions can significantly affect bacterial surface properties, including charge and hydrophobicity *(3, 10, 19)*. Ukuku and Fett *(21)* previously concluded that bacterial cell surface charge and hydrophobicity appear to be highly correlated with the strength of attachment to the melon surface. According to Fletcher *(13)*, bacterial adhesion occurs in three steps: reversible adsorption, primary adhesion, and colonization. During the reversible adsorption phase, the bacterium is more than 50 nm away from the substratum and is affected by van der Waal interactions with the substratum. According to Buscher et al. *(5)*, once bacteria overcome the water barrier and a separation distance of less than 1 nm exists, additional adhesion factors such as hydrogen bonding, cation bridging, and receptor-ligand interactions between bacteria and plant surfaces become important. At this stage, the bacteria are very difficult to remove by washing. At the primary adhesion stage, the distance between the bacteria and the substratum ranges from 10 to 20 nm, and the type of force affecting adhesion is electrostatic unless the opposing surface has a net surface charge, when attractive forces then come into play. The colonization step is the final phase for firm bacterial attachment, and at this point biofilms may be formed and make killing of bacteria by chemical means difficult. Liao and Sapers *(14)* reported rapid attachment of *Salmonella* to apple tissue and the inability of sanitizer treatment

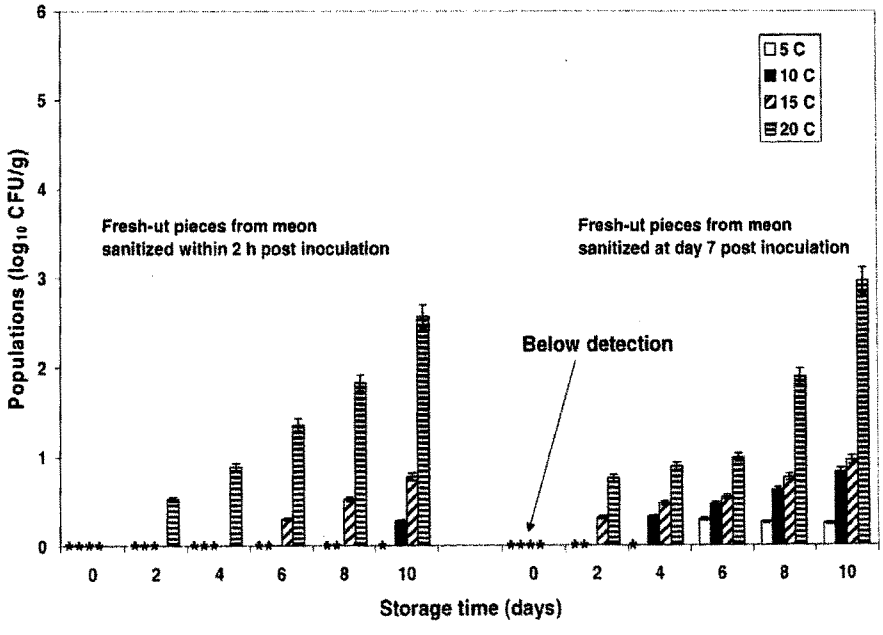

FIGURE 3. *Populations of a five-strain cocktail of* Salmonella *Poona on fresh-cut pieces prepared from inoculated cantaloupe sanitized with chlorine (200 ppm) at day 0 (A) or day 7 (B) postinoculation. Fresh-cut pieces were stored at 5 to 20°C for up to 10 days. Values are mean ± SD of three experiments, with duplicate determinations per experiment. *Below detection limit (<1 CFU/g).*

to inactivate the attached pathogen. Storage at 25°C simulated contamination of cantaloupe under natural conditions in the field, where production of cellulose and curli by *Salmonella (4, 30)* may allow the bacterium to strongly bind to the plant surface and become highly resistant to removal by rain or by washing steps during processing.

Salmonella was not detected in fresh-cut pieces prepared from melon sanitized immediately after inoculation regardless of the day of sanitizer application to the whole melons (Fig. 3). However, when fresh-cut pieces were obtained from melons stored at 5, 10, 15, and 20°C for up to 10 days, transferred pathogens were detected in samples sanitized at 7 days postinoculation, indicating a reduced ability of chlorine to eliminate the pathogen from the contaminated cantaloupe surface after prolonged storage. This result is consistent with those of earlier studies that revealed decreased antimicrobial activity of chlorine or hydrogen peroxide against native bacteria and *E. coli* on whole cantaloupe surfaces after storage for 2 days or longer *(22)*.

The results of this study clearly indicate that investigations of antimicrobial activities of sanitizers on contaminated melons should factor in storage time and temperature before applying the treatments because point of contamination is not generally known and in most cases may precede washing by days or weeks. It is important to maintain the cold chain after fresh-cut preparation of sanitized fruit to inhibit the growth of any surviving pathogens transferred to the fresh-cut pieces and to reduce the risk of enteric disease. Although *Salmonella* Michigan (cantaloupe-related strain) exhibited the strongest attachment at several of the sampling times, cantaloupe-related strains in general did not attach more strongly to the cantaloupe rind than did non–cantaloupe-related strains.

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REFERENCES

1. Andrews, W. H., V. R. Bruce, G. June, F. Satchell, and P. Sherrod. 1995. *Salmonella*, p. 5.01–5.19. *In* Bacteriological analytical manual, 8th ed. Association of Official Analytical Chemists, Gaithersburg, Md.
2. Annous, B. A., A. Burke, and J. E. Sites. 2004. Surface pasteurization of whole fresh cantaloupes inoculated with *Salmonella* Poona or *Escherichia coli*. *J. Food Prot.* 67:1876–1885.
3. Briandet, R., T. Meylheuc, C. Maher, and M. N. Bellon-Fontaine. 1999. *Listeria monocytogenes* Scott A: cell surface charge, hydrophobicity, and electron donor and acceptor characteristics under different environmental growth conditions. *Appl. Enivron. Microbiol.* 65:5328–5333.
4. Brown, P. K., C. M. Dozois, C. A. Nickerson, A. Zuppardo, J. Terlonge, and R. Curtiss III. 2001. MirA, a novel regulator of curli (AgF) and extracellular matrix synthesis by *Escherichia coli* and *Salmonella enterica* serovar Typhimurium. *Mol. Microbiol.* 41:349–363.
5. Buscher, H. J., J. Sjollema, and H. C. Van der Mei. 1990. Relative importance of surface free energy as a measure of hydrophobicity in bacterial adhesion to solid surfaces, p. 335. *In* R. J. Doyle and M. Rosenberg (ed.), Microbial cell surface hydrophobicity. American Society for Microbiology, Washington, D.C.
6. Castillo, A., L. Mercado, L. M. Lucia, Y. Martínez-Ruiz, J. Ponce de León, E. A. Murano, and G. R. Acuff. 2004. *Salmonella* contamination during production of cantaloupe: a binational study. *J. Food Prot.* 67:713–720.
7. Centers for Disease Control and Prevention. 2002. Multistate outbreaks of *Salmonella* serotype Poona infections associated with eating cantaloupe from Mexico—United States and Canada, 2000–2002. *Morb. Mortal. Wkly. Rep.* 51:1044–1047.
8. Dahlback, B., M. Hermansson, S. Kjelleberg, and B. Norkrans. 1981. The hydrophobicity of bacteria—an important factor in their initial adhesion at the air-water interface. *Arch. Microbiol.* 128:267–270.
9. Dewaal, C. S., and K. Barlow. 2002. Outbreak alert! Closing the gaps in our federal food safety net. Center for Science in the Public Interest, Washington, D.C. Available at: http://cspinet.org/foodsafety/reports.html. Accessed 16 June 2003.
10. Dewanti, R., and A. C. L. Wong. 1995. Influence of culture conditions on biofilm formation by *Escherichia coli* O157:H7. *Int. J. Food. Microbiol.* 26:147–164.
11. Dickson, J. S., and M. Koohmaraie. 1989. Cell surface charge characteristics and their relationship to bacterial attachment to meat surfaces. *Appl. Environ. Microbiol.* 55:832–836.
12. Fett, W. F. 1985. Relationship of bacterial cell surface hydrophobicity and charge to pathogenicity, physiologic race, and immobilization in attached soybean leaves. *Phytopathology* 75:1414–1418.
13. Fletcher, M. 1996. Bacterial attachment in aquatic environments: a diversity of surfaces and adhesion strategies, p. 1–24. *In* M. Fletcher (ed.), Bacterial adhesion: molecular and ecological diversity. John Wiley and Sons, Inc., New York.
14. Liao, C.-H., and G. M. Sapers. 2000. Attachment and growth of *Salmonella* Chester on apple fruits and in vivo response of attached bacteria to sanitizer treatments. *J. Food Prot.* 63:876.
15. Miller, R. G., Jr. 1981. Simultaneous statistical inference, 2nd ed., p. 67–70. Springer-Verlag, New York.
16. Pedersen, K. 1980. Electrostatic interaction chromatography, a method for assaying the relative surface charge of bacteria. *FEMS Microbiol. Lett.* 12:365–367.
17. Romantschuk, M. 1992. Attachment of plant pathogenic bacteria to plant surfaces. *Annu. Rev. Phytopathol.* 30:225–243.
18. Romantschuk, M., E. Roine, K. Bjorklof, T. Ojanen, E.-L. Nurmiaho-Lassila, and K. Haahtela. 1996. Microbial attachment to plant aerial surfaces, p. 43–57. *In* C. E. Morris, P. C. Nicot, and C. Nguyen-The (ed.), Aerial plant surface microbiology. Plenum Press, New York.
19. Sherman, P., R. Soni, M. Petric, and M. Karmali. 1987. Surface properties of Vero cytoxin–producing *Escherichia coli* O157:H7. *Infect. Immun.* 55:1824–1829.
20. Ukuku, D. O. 2004. Effect of hydrogen peroxide treatment on microbial quality and appearance of whole and fresh-cut melons contaminated with *Salmonella* spp. *Int. J. Food Microbiol.* 95:137–146.
21. Ukuku, D. O., and W. F. Fett. 2002. Relationship of cell surface charge and hydrophobicity to strength of attachment of bacteria to cantaloupe rind. *J. Food Prot.* 65:1093–1099.
22. Ukuku, D. O., V. Pilizota, and G. M. Sapers. 2001. Influence of washing treatments on native microflora and *Escherichia coli* population of inoculated cantaloupes. *J. Food Saf.* 21:31–47.
23. Ukuku, D. O., V. Pilizota, and G. M. Sapers. 2004. Effect of hot water and hydrogen peroxide treatment on survival of *Salmonella* and microbial quality of whole cantaloupe and fresh-cut cantaloupe. *J. Food Prot.* 67:432–437.
24. Ukuku, D. O., and G. M. Sapers. 2002. Effect of sanitizer treatments on *Salmonella* Stanley attached to the surface of cantaloupe and cell transfer to fresh-cut tissues during cutting practices. *J. Food Prot.* 64:1286–1291.
25. U.S. Food and Drug Administration. 25 May 2001. Produce safety at retail: safe handling practices for melons. U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition. Available at: http://www.cfsan.fda.gov/~ear/ret-mlm.html. Accessed 12 May 2003.
26. U.S. Food and Drug Administration. 2001. FDA survey of imported

fresh produce. U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition. Available at: http://www.cfsan.fda.gov/~dms?prodsur6.html. Accessed 16 June 2003.

27. U.S. Food and Drug Administration. 2003. FDA survey of domestic fresh produce. U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition. Available at: http://www.cfsan.fda.gov/~dms/prodsu10.html. Accessed 6 August 2004.
28. U.S. Food and Drug Administration. 2003. Detention without physical examination of cantaloupes from Mexico. Import alert IA2201 attachment. Available at: http://www.fda.gov.ora/fiars/ora_import_ia2201.html. Accessed 6 August 2004.
29. Webster, B. D., and M. E. Craig. 1976. Net morphogenesis and characteristics of the surface of muskmelon fruits. *J. Am. Soc. Hort. Sci.* 101:412–415.
30. Zogaj, X., M. Nimtz, M. Rohde, W. Bokranz, and U. Romling. 2001. The multicellular morphotypes of *Salmonella typhimurium* and *Escherichia coli* produce cellulose as the second component of the extracellular matrix. *Mol. Microbiol.* 39:1452–1463.